0022-202X/81/7703-0314$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 77:314–318, 1981
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Vol. 77, No. 3
Printed in U.S.A.

Experimental Infections in Rabbits and Humans with *Pityrosporum orbiculare* and *P. ovale*

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The purpose of this investigation was to produce experimental tinea versicolor in rabbits and humans with *Pityrosporum orbiculare* and *P. ovale*.

***P. orbiculare* and *P. ovale* were inoculated, with and without occlusion, on the inside of the ear in 10 male rabbits and on the upper arm in 10 patients with a history of tinea versicolor, and in 3 healthy volunteers.**

After 1 week tinea versicolor-like lesions were produced with both *P. orbiculare* and *P. ovale* in 8 of 10 rabbits. Likewise experimental infections, similar to those found clinically in tinea versicolor, were seen, after 1 week, in 5 patients with a history of tinea versicolor and in the 3 healthy volunteers. Two of 5 patients inoculated for only 4 days showed identical but less pronounced lesions. Experimental infections could only be produced with occlusion. Microscopically short hyphae and transformation between round and oval forms were seen in both *P. orbiculare* and *P. ovale*.

This investigation adds to the identity of *P. orbiculare* and *P. ovale* and also to the identity of these 2 fungi and the fungus seen in tinea versicolor. Spontaneous healing and the fact that experimental infections were produced only under occlusion illustrates the importance of predisposing factors in tinea versicolor.

The lipophilic yeasts *Pityrosporum orbiculare* and *P. ovale* are members of the normal human cutaneous flora [1–6]. *P. orbiculare* is not only a saprophyte but also the probable etiological agent of tinea versicolor [1,7–9]. In tinea versicolor *P. orbiculare* is thought to change from its saprophytic yeast phase to its pathogenic mycelial phase [7,10]. There have even been reports of the production of hyphae of *P. orbiculare in vitro* in culture media with glycine [11] and cholesterol and cholesterol esters [12]. *P. ovale* has earlier been associated with seborrheic dermatitis [5] and there have been reports of the production of seborrheic dermatitis-like lesions with this fungus [13–15]. The fungi described in some of these reports [13] could later not be identified as *P. ovale*. Today *P. ovale* is not regarded as the etiological agent of seborrheic dermatitis [3,16–18]. The role of *P. orbiculare* and *P. ovale* in acne and folliculitis is still debated; some workers regard them as saprophytes [19] and others as pathogens [20,21].

In recent years there has been an increasingly intense discussion as to the question of the identity of *P. orbiculare* and *P. ovale* [11,12,22–28].

In an earlier investigation we were able to produce experimental tinea versicolor in rabbits and humans with *P. orbiculare* under occlusion [7]. In view of this result, and because the problem of the identity of *P. orbiculare* and *P. ovale* is still unsolved, an attempt to produce experimental infections with *P. orbiculare* and *P. ovale* under occlusion was made.

Manuscript received October 27, 1980; accepted for publication February 10, 1980.

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Abbreviations:
PBS: phosphate buffered saline

MATERIALS AND METHODS

Fungi

One strain of *P. orbiculare*, number 0639/77 from our own collection, and a strain of *P. ovale* from the American Type Culture Collection (ATCC), number 1452, were used. The *P. orbiculare* strain was primarily cultured from tinea versicolor lesions and showed germ tubes in primary cultures, but not in the secondary culture used for inoculation. Both *P. orbiculare* and *P. ovale* were grown on a medium containing neopeptone (Difco) 10 g/l, Bacto agar (Difco) 18 g/l, glucose 40 g/l, yeast extract (Difco) 0.1 g/l, glycerol monostearate 2.5 g/l, Tween 80 2 ml/l, and olive oil 20 ml/l; pH adjusted to 6.0. After autoclave sterilization chloramphenicol (50 mg/l), gentamycin (100 mg/l), and actadione mixture 1.25 ml/l (0.5 g actadione, 2 ml acetone, and 10 ml aqua dest.) were added. The plates were incubated aerobically at 37°C for 4 days.

Preparation of Fungi and Additive Solutions

In some experiments 1 μl of a pure culture of *P. orbiculare* and *P. ovale*, from 4-day-old cultures, was used as inoculum. The cells were taken, with an ordinary loop, directly from the culture plate (mean: 1.7×10^7 cells, as determined in a counting chamber).

In other experiments the result of using cells suspended in phosphate buffered saline (PBS) (10^8 cells) and the effect of adding olive oil or a mixture of cholesterol, cholesteryl stearate, and glyceryl monostearate (2:1.5:2) to the inoculum were studied.

Inoculation of P. orbiculare *and* P. ovale *in Rabbits*

Ten male New Zealand White rabbits weighing 2.8–3.2 kg were used. The inoculation area was the unshaven inside of the ear. One week prior to inoculation superficial skin scrapings for culture were taken from the inside of both ears in all rabbits with the aid of a curette. The specimens were transferred to the above mentioned medium and to a usual Sabouraud's medium (Difco). This was done to eliminate the possibility of other fungi being present, especially *P. pachydermatis*, another nonlipophilic member of the genus *Pityrosporum* which is found primarily in animals. In addition specimens were taken for aerobic culture of bacteria on blood agar. The plates were incubated at 37°C. Immediately before inoculation the skin area was investigated clincally and under Wood's light, and only skin of normal appearance and without fluorescence in Wood's light was chosen.

Experimental design: Five rabbits were inoculated as follows: *Right ear. P. orbiculare* strain no. 0639/77 was used, and inoculation was done by 3 methods:

1. Inoculation of a pure culture, taken directly from the culture plate with an ordinary loop, on an area 1 × 1.5 cm on the inside of the ear. The area was occluded with an 1 × 1.5 cm plastic film (Scott plastic wrap, U.S.A.). This was held in place by Scanpor tape (Scanpor, Norgesplaster A/S, Norway) covered with Leukoplast tape (Beiresdorf, Germany).
2. Approximately 3–4 cm away from the above mentioned area an area 1 × 1.5 cm was occluded, using the same technique as in 1, but without any fungi.
3. Inoculation of *P. orbiculare* without occlusion on the peripheral edge.

b. *Left ear.* Inoculation was done the same way as described under point a but instead of *P. orbiculare*, strain no. 1452 of *P. ovale* was used.

In 5 other rabbits 10^8 cells of *P. orbiculare* and *P. ovale* suspended in PBS, with or without the addition of the cholesterol, cholesteryl stearate, and glyceryl monostearate mixture, were used as inoculum. *P. orbiculare* was inoculated on the right ear and *P. ovale* on the left ear.

The inoculation period in all experiments was 7 days. The inoculated site was then investigated as follows: clinically, under Wood's light, microscopically, and by skin scrapings for culture. For microscopic

TABLE I. *Results of inoculation of* Pityrosproum orbiculare *(*P. orb.*) and* P. ovale *(*P. ov.*) on the inside of the ear in rabbits after 1 week*

Technique	Rabbit No.	Number of positive findings							
		Lesions		Wood's light		Microscopy		Culture	
		P. orb.	*P. ov.*	*P. orb*	*P. ov.*	*P. orb.*	*P. ov.*	*P. orb.*	*P. ov.*
10^8 Cells in PBS inoculated under occlusion	5	3	3	3	3	3	3	5	5
Cells inoculated directly from the culture plate under occlusion	5	5	5	5	5	5	5	5	5
Occlusion without *P. orb.* or *P. ov.*	5	None							
Inoculation without occlusion	5	None							

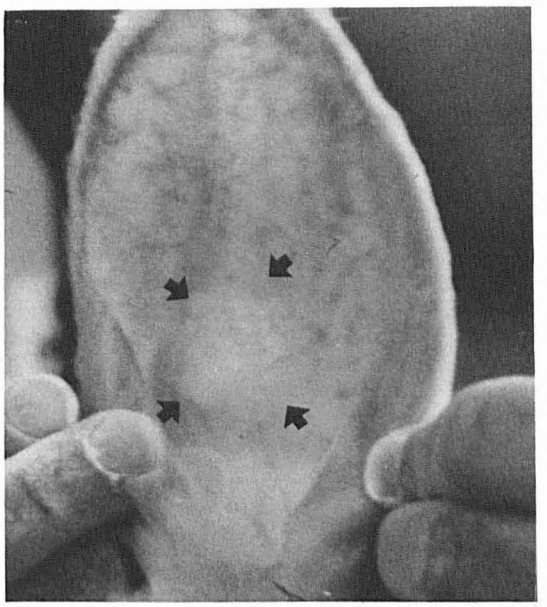

FIG 1. Experimental infection on the rabbit ear after 1 week of occlusion with *Pityrosporum orbiculare* (identical with *P. ovale*) (Wood's light).

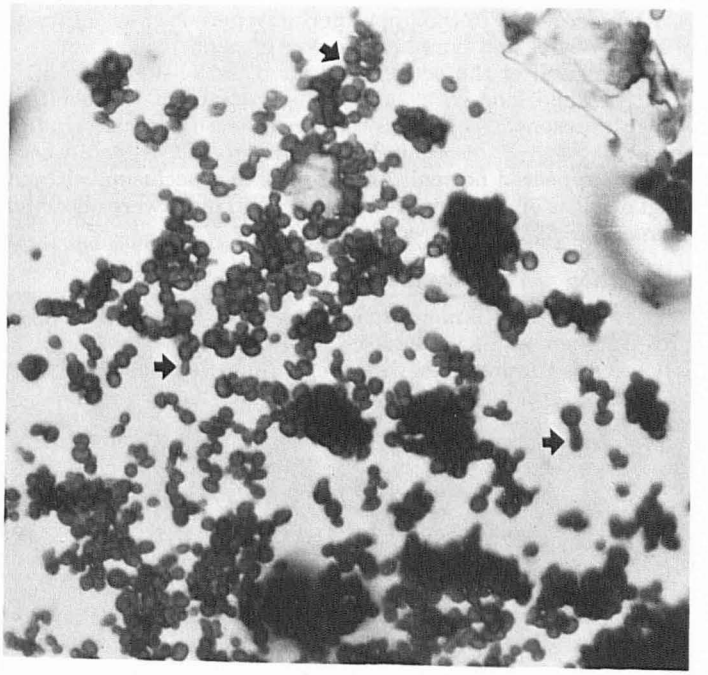

FIG 2. Microscopic view of experimental infection in rabbits with *Pityrosporum orbiculare* showing round-, budding cells and short hyphae (reduced from × 400).

studies thin layers of epidermal cells were obtained with Scotch tape. The tape was stained with methylene blue (1%, 1 min) for light microscopy. In addition a punch biopsy was taken from both ears in 6 rabbits. The inoculation areas were investigated at weekly intervals. Five rabbits were reoccluded, after 2 weeks, on both ears, and on the same sites, but without fungi. The areas were marked with a pen.

Inoculation of P. orbiculare *and* P. ovale *in Humans*

Ten patients with a past history of tinea versicolor and 3 normal healthy volunteers, without any known skin diseases, participated in the inoculation experiment. All of the subjects were in good health and inoculation was done in the winter or spring time. The inoculation sites were on skin of normal appearance on the lateral aspect of the upper arm. The patients had been cured of tinea versicolor at different intervals before inoculation, and they had no sign of tinea versicolor when judged clinically, under Wood's light, and microscopically. None of the patients had had tinea versicolor on the arms, and the inoculation area had not been treated. Before inoculation the site and its surroundings were investigated clinically and under Wood's light. The strains of *P. orbiculare* and *P. ovale* used were the same as in the rabbits, but only cells taken with a loop directly from the culture plate were used. The occlusion technique was also identical but instead of Leukoplast tape, tube gauze (School AB, Stockholm, Sweden) was used to cover the occlusive bandage. The inoculation area was 1 × 1.5 cm and the different areas were approximately 2–3 cm apart.

Experimental Design

1. Five patients were inoculated with *P. orbiculare* and *P. ovale* and occluded for 4 days without additives or controls.

2. Five patients were inoculated with *P. orbiculare* and *P. ovale* for 7 days. In this experiment 5 different techniques were used for both *P. orbiculare* and *P. ovale*: (a) The fungi and occlusion. (b) The fungi, occlusion, and 1 μl of sterile olive oil. (c) The fungi, occlusion, and 1 μl of a 2% aquous mixture of cholesterol, cholesteryl stearate, and glyceryl monostearate (2:1.5:2). (d) Two areas were inoculated once with either *P. orbiculare* or *P. ovale* but without occlusion. (e) As a control occlusion with plastic film but without *P. orbiculare* or *P. ovale*.

3. Three normal volunteers, who never had tinea versicolor, were inoculated as described under point 2.

After the inoculation period the areas were examined clinically, under Wood's light, microscopically, and skin scrapings were taken for culture. A punch biopsy was taken from 2 volunteers. The patients were seen again after 4 weeks. In the volunteers the inoculation sites were examined daily for 3 weeks.

RESULTS

Inoculation of P. orbiculare and P. ovale in rabbits.

Cultures taken 1 week prior to inoculation were negative for *P. orbiculare*, *P. ovale*, *P. pachydermatis*, and other fungi. The bacteria found were the normal residents of the skin. Prior to inoculation no fluorescence was seen in Wood's light. The results after 1 week are shown in Table I. All of the 5 rabbits inoculated with *P. orbiculare* and *P. ovale* cells taken directly from the culture plate under occlusion, and in 3 of 5 rabbits where PBS-suspended cells were inoculated under occlusion a red-yellow-brown scaling lesion with a bright yellow fluorescence in Wood's light appeared after 7 days (Fig 1). No differences were observed between *P. orbiculare* and *P. ovale*. The criteria for positive microscopy were, besides round and budding cells, the presence of short hyphae (Fig 2 and 3). To describe the result of culture a semiquantitative technique was used: +++: growth on more than 50% of the plate, ++: growth on 10–50% of the plate, +: growth on less than 10% of the plate, −: no growth. Growth in culture was more luxurious from

rabbits inoculated with cells taken directly from the culture plate (+++) than when cells suspended in PBS were used (+ in 2, ++ in 2, and +++ in only 1). When yeast cells suspended in PBS were used the lesions were also less obvious than when cells taken directly from the culture plate were used. However, the PBS-suspended yeasts showed a high viability with a loss of less than 10% when colony forming units were counted. No differences were observed when inoculation was done with a mixture of cholesterol, cholesteryl stearate, and glyceryl monostearate added to the fungal inoculum. Inoculation of both *P. orbiculare* and *P. ovale* showed histologically (PAS-staining) a mixture of round, oval, and short elongated cells in the stratum corneum. In the upper dermis a perivascular infiltrate of granulocytes and lymphocytes was present.

One week after the occlusive bandage had been taken off a slight scaling lesion with a slight fluorescence in Wood's light was still present. A few fungi were seen microscopically and *P. orbiculare* and *P. ovale* could still be cultured. After 2 weeks the skin appeared normal. After 7 days of reocclusion without fungi no signs of infection were seen and no fungi were observed microscopically or cultured.

Inoculation of P. orbiculare *and* P. ovale *in Humans*

The results are summarized in Table II. Two of the 5 patients with a history of tinea versicolor showed after 4 days a slight yellow-brown lesion with weak yellow fluorescence in Wood's light. No differences were seen between *P. orbiculare* and *P. ovale*. Microscopically short hyphae were present. All of the patients and healthy volunteers inoculated for 7 days and occlusion showed slight scaling red-yellow-brown lesions with a bright yellow fluorescence in Wood's light (Fig 4). No differences were observed with occlusion alone, occlusion and olive oil, or occlusion and the cholesterol mixture. Identical lesions were seen with *P. orbiculare* and *P. ovale*. Microscopically the same picture as in the rabbits was seen. In both humans and rabbits it was not always possible to observe any morphological differences between specimens of *P. orbiculare* and *P. ovale*, except that *P. ovale* cells were larger (Fig 5 and 6). The histological picture was essentially the same as in the rabbits.

When the 10 patients with a history of tinea versicolor were seen after 4 weeks no signs of infections were present. In the volunteers no signs of infection were present 1 week after termination of occlusion.

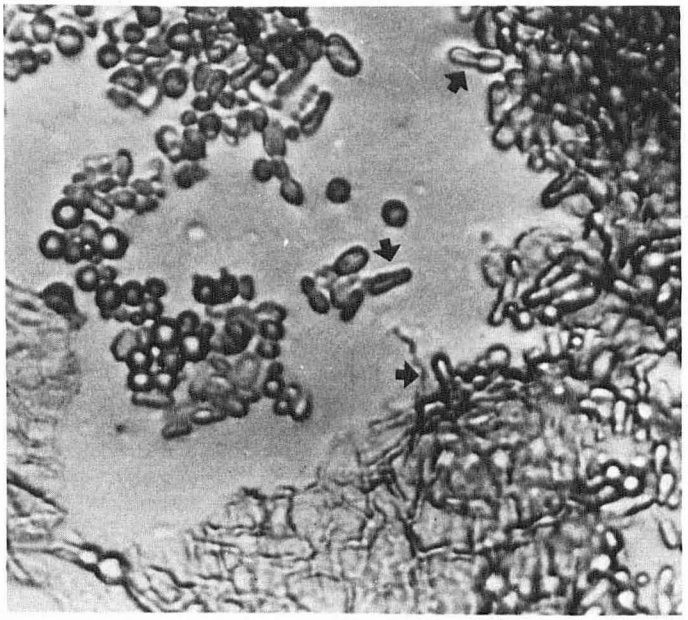

FIG 3. Microscopic view of experimental infection in rabbits with *Pityrosporum ovale* showing oval-, budding cells, and short hyphae (reduced from × 400).

DISCUSSION

Are *P. orbiculare* and *P. ovale* different evolution forms of the same yeast or are they altogether different species? Geographically differences in occurrence of *P. orbiculare* and *P. ovale* are found. Both in Sweden [7,29] and Italy [27] the *P. ovale* form is not, or only seldom, cultured. In England and the U.S.A. both forms are cultured, and there the greatest incidence of *P. ovale* is found on the scalp and *P. orbiculare* on the back [4,16]. Usually the *P. orbiculare* form is cultured from tinea versicolor lesions [1,3,7] but the culture of *P. ovale* has also been reported [30]. No differences are observed in the macromorphology of *P. orbiculare* and *P. ovale* and fermentation tests are negative in both [31]. They are both lipophilic but only *P. ovale* can be maintained on Littmans oxgall agar without the addition of further lipids [31]. The micromorphology is quite different in *P. orbiculare* and *P. ovale* [1,28], but there have been reports of the change of *P. orbiculare* from globose through ovoid to cylindroid form [22,23]. Antigenic similarities between the 2 fungi have been described [24,25], but in other reports antigenic differences have been found [9,26]. Porro et al reported in 1977 the production of identical hyphae *in vitro* in both *P. orbiculare* and *P. ovale* using a culture medium with a mixture of cholesterol and cholesterol esters [12]. Dorn and Roehnert were also able to produce hyphae in *P. orbiculare*, but not in *P. ovale*, using a culture medium containing glycine [11]. Attempts to produce experimental infections in rabbits with *P. orbiculare* have earlier failed [1]. However, Drouhet and Dompmartin recently claimed to have produced lesions similar to human seborrheic dermatitis in shaved guinea pigs and rats with both *P. orbiculare* and *P. ovale*, but failed to do so in hairless mice [14,15]. The lesions were not examined under UV-light but contained yeast cells and no hyphae. We have never observed lesions of this type in our animals, volunteers, patients with active tinea versicolor, or confluent and reticulate papillomatosis of Gougerot-Carteaud [32].

TABLE II. *Results of inoculation of* Pityrosporum orbiculare *(*P. orb.*) and* P. ovale *(*P. ov.*) on the upper arm in patients with a history of tinea versicolor and healthy volunteers after 4 days and 1 week*

Technique	Patient No.	Number of positive findings							
		Lesions		Wood's light		Microscopy		Culture	
		P. orb.	*P. ov.*	*P. orb.*	*P. ov.*	*P. orb.*	*P. ov.*	*P. orb.*	*P. ov.*
Inoculation for 4 days and occlusion	5	2	2	2	2	2	2	5	5
Inoculation for 7 days:									
Inoculation under occlusion	5	5	5	5	5	5	5	5	5
Occlusion without *P. orb.* or *P. ov.*	5					None			
Inoculation without occlusion	5					None			
	Volunteer No.								
Inoculation under occlusion	3	3	3	3	3	3	3	3	3
Occlusion without *P. orb.* or *P. ov.* and inoculation without occlusion	3					None			

We have in an earlier investigation produced experimental tinea versicolor in both rabbits and humans with *P. orbiculare*. When we used this model we succeeded in producing macroscopically identical experimental infections in rabbits and humans with *P. orbiculare* and *P. ovale*. Experimental infections could only be produced under occlusion. Occlusion raises the temperature, humidity, and CO_2 tension [33,34] and these are factors known to be predisposing in tinea versicolor [3,7,8].

Microscopically short identical hyphae were found in lesions produced by both *P. orbiculare* and *P. ovale* and even conversion between round and oval forms were observed. The lesions showed a bright yellow fluorescence in Wood's light while the negative control inoculation sites, olive oil, or the cholesterol mixture did not. Although yellow fluorescence in Wood's light is not specific for tinea versicolor it is of great diagnostic help in this disease. It is also a sign of conversion of *P. orbiculare* (*P. ovale*) from saprophyte to pathogen because pure cultures of *P. orbiculare* and *P. ovale* do not show fluorescence in Wood's light [6].

In 1977 Porro et al reported the successful production of identical hyphae *in vitro* in *P. orbiculare* and *P. ovale* when they were grown in a culture medium containing a mixture of cholesterol, cholesteryl stearate, and glyceryl monostearate [12]. In our experiments no differences were observed in the experimental infections with or without this additive.

As might be suspected from other inoculation experiments the induced infections healed spontaneously. In the earlier study of experimental tinea versicolor with *P. orbiculare* we reported one case where we succeeded in reproducing the experimental infection, after it had healed, with occlusion alone. When we now tried to do this in 5 rabbits we were unsuccessful, but this may be due to too long a period before the reocclusion was done.

This investigation adds to the identity of *P. orbiculare* and *P. ovale* but other experiments are needed to finally confirm this identity.

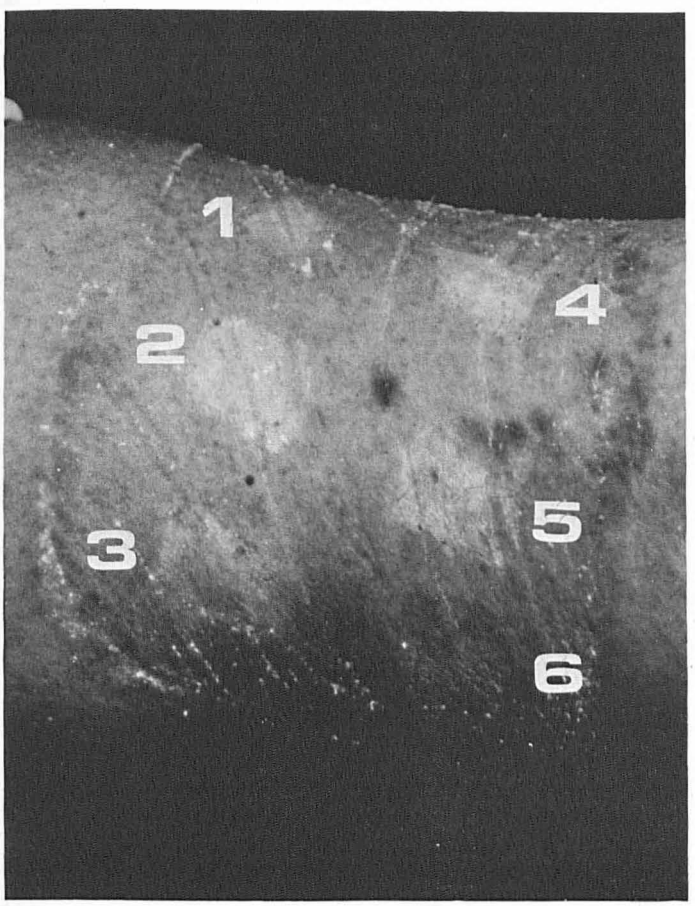

FIG 4. Experimental infections in a human after 1 week of occlusion with *Pityrosporum orbiculare* (right, *4-6*), and *P. ovale* (left, *1-3*) (Wood's light). *One* and *4* are occlusion with fungi alone. *Two* and *5* are occlusion with fungi and a mixture of cholesterol, cholesterol stearate, and glyceryl monostearate. *Three* and *6* are occlusion with fungi and sterile olive oil (the indistinct appearance of No. 6 is due to the angle of the light).

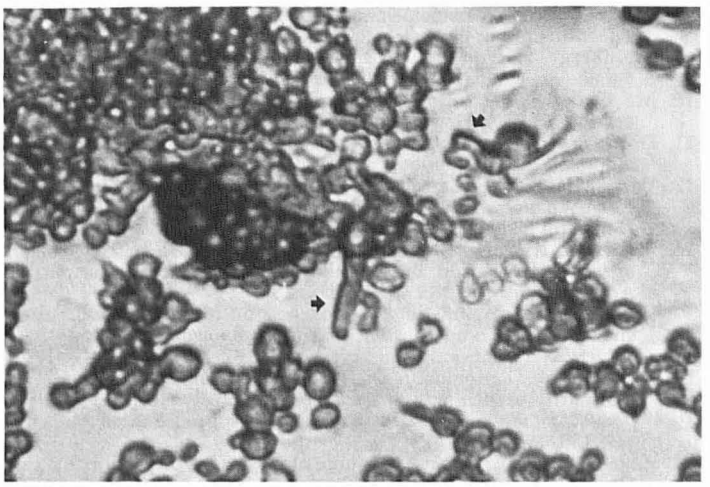

FIG 5. Microscopic view of experimental infections with *Pityrosporum orbiculare* in humans showing conversion from round to oval forms and in addition short hyphae (reduced from × 500).

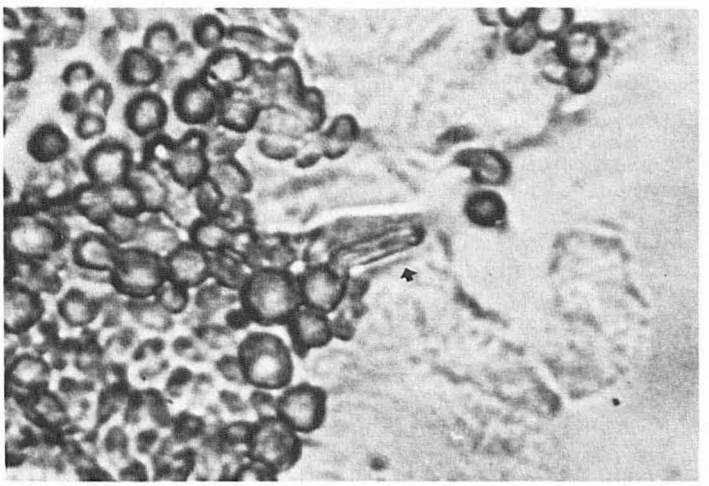

FIG 6. Microscopic view of experimental infections with *Pityrosporum ovale* in humans showing conversion from oval to round forms and in addition short hyphae (reduced from × 500).

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